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THE GROWTH OF RIFT VALLEY FEVER VIRUS IN CULTURES OF ESTABLISHED LINES OF CELLS

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THE virus of Rift Valley fever (RVF) has been grown in a variety of tissue culture systems. The culture systems that have been reported to support the growth of this virus include minced chick embryo in Tyrodes solution;^{8,12} embryonic mouse brain;² rat sarcoma cells, human embryo, rat, mouse, and swine fibroblasts;^{15,17} ovine kidney cells;^{8,18} monkey kidney cells;⁹ and Chang's human liver cells.⁴

The purpose of this report is to record the ability of five established lines of cells to support the growth of Rift Valley fever virus (RVFV).

MATERIALS AND METHODS

Virus strain. The van Wyk strain of RVFV was used. Kaschula⁵ described its isolation from the blood of a sheep from the Boshof District of the Orange Free State in the Union of South Africa. The working seed virus (lamb serum) used in these studies had a titer of $1 \times 10^{9.5}$ mouse intraperitoneal median lethal doses (MIPLD₅₀) per ml. All material that contained virus was assayed by the intraperitoneal (IP) inoculation of mice (Swiss-Webster strain, 10 to 14 grams

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in weight or 21 to 28 days old). The selection of this method of assay was based on the information available in the literature on the sensitivity of mice to RVFV. The mice were observed for 5 days after inoculation, and the median lethal dose was calculated by the method of Reed and Muench.¹⁰

Cell lines. Five established cell lines, Chang's human liver (CHL) cells,¹ HeLa cells,¹⁴ guinea pig lung (GPL) cells, and hamster kidney (HK) cells were utilized in these studies. The first three lines were from cultures maintained at Fort Detrick. The GPL and HK cells were lines that were established and maintained at Fort Detrick.

All of the cultures were grown as monolayers at 37 C in T-30 or T-60 tissue culture flasks utilizing medium 199⁷ pH 7.4 to 7.6 with 10 percent calf serum. Penicillin (250 μ per ml) and dihydrostreptomycin (250 μ g per ml) were added to the medium. When confluent monolayers were obtained, the cells were infected with the desired amount of virus in the following manner: The cells were washed once with Hank's balanced salt solution (BSS),³ and the desired amount of virus suspension was placed on the cells and allowed to absorb at 37 C for 1 hour. After the absorption period the monolayers were washed three times with BSS, and growth medium was placed on the cells (6 ml for the T-30 flasks and 15 ml for the T-60 flasks). The medium was sampled 30 minutes after it was placed on the cells and then daily for 5 to 7 days. Unless otherwise indicated, all cultures were held at 37 C. Sampling consisted of taking 0.8 ml of medium from the flask and adding it to 7.2 ml of Bacto heart infusion broth (BHIB-Difco). This diluted material was frozen at -30 C until it was assayed.

The cells were observed daily for evidence of destruction by the virus. Evaluation was based on comparison with cells handled in the same manner but not exposed to virus. The degree of cellular destruction, or cytopathogenic effect (CPE), was graded as 1, 2, 3, or 4+ CPE.

RESULTS

Cultures of L cells were inoculated with $2 \times 10^{3.5}$ and $2 \times 10^{4.0}$ MIPLD₅₀ of RVFV in two experiments. In the first

trial a peak titer of $1 \times 10^{3.5}$ MIPLD₅₀ per ml of culture fluid was demonstrated 24 hours after inoculation. In the second trial the peak titer was $1 \times 10^{3.9}$ 72 hours after inoculation (table I). No CPE could be attributed to the virus.

TABLE I

Growth of Rift Valley fever virus (RVFV) in L cell monolayers at 37 C

Day	Experiment I			Experiment II		
	Control*	Infected†		Control	Infected ‡	
	CPE§	CPE	MIPLD ₅₀	CPE	CPE	MIPLD ₅₀
0			Neg#			Neg
1			3.8			3.2
2			3.5			3.7
3			3.2			3.9
4	1+	1+	3.5			2.3
5	1+	1+	2.8	1+	1+	3.5

*Control flask—Not inoculated with RVFV. Undiluted medium not lethal for mice.

†Cell culture inoculated with $2 \times 10^{3.5}$ MIPLD₅₀ of RVFV.

‡Cell culture inoculated with $2 \times 10^{4.0}$ MIPLD₅₀ of RVFV.

§CPE—Cytopathogenic effect.

||MIPLD₅₀—Log₁₀ mouse intraperitoneal median lethal doses per ml of culture medium (15 ml culture medium per flask).

#Neg—Virus could not be demonstrated in 1:10 dilution of culture medium.

No attempts were made to infect L cells with greater amounts of viral material.

CHL cells were inoculated with $2 \times 10^{3.5}$ and $2 \times 10^{4.5}$ MIPLD₅₀ of RVFV and incubated at 34 C and 37 C. In those cultures inoculated with the larger dose the peak titer of virus in the culture medium was slightly higher and was attained earlier in the cultures incubated at 37 C than in those incubated at 34 C. Complete destruction of cells also occurred earlier in the cultures incubated at 37 C (table II). In those cultures inoculated with the smaller dose the peak titer was the same at each temperature of incubation. However, the peak titer and complete destruction of the cells did occur 1 day earlier in the cultures incubated at 37 C. At each temperature of incubation complete destruction of cells occurred 1 day earlier in the cultures inoculated with $2 \times 10^{3.5}$ MIPLD₅₀.

The infecting dose did not appear to influence significantly the peak titer of virus in the culture medium. In the cultures incubated at 37 C, however, the peak titer was on the 1st day after inoculation in the cultures that received $2 \times 10^{6.5}$ MIPLD₅₀ of RVFV and on the 3rd day in the cultures that received $2 \times 10^{6.5}$ MIPLD₅₀ (table II).

TABLE II

Growth of Rift Valley fever virus (RVFV) in Chang human liver (CHL) cell monolayers

Day	Control*	Cells inoculated with $2 \times 10^{6.5}$ MIPLD ₅₀		Cells inoculated with $2 \times 10^{6.5}$ MIPLD ₅₀	
	CPE	Virus/ml†	CPE‡	Virus/ml	CPE
<i>Incubated at 34 C</i>					
0		4.1		2.5	
1		6.4	1+	5.7	
2		6.5	3+	6.7	2+
3		6.2	3+	6.6	2+
4		6.8	4+	7.2	3+
5	1+	6.2	4+	6.2	4+
<i>Incubated at 37 C</i>					
0		4.3		2.5	
1		7.4	2+	4.8	
2		7.2	3+	6.5	2+
3		5.8	4+	7.2	3+
4		6.3	4+	6.8	4+

*Control flask—Not inoculated with RVFV. Undiluted medium not lethal for mice (IP).

†Log₁₀ MIPLD₅₀ of RVFV per ml of cell culture medium (15 ml medium per flask).

‡CPE—Cytopathogenic effect, degree of cell destruction.

HeLa cells were inoculated with three different levels of virus (table III). The results obtained when $2 \times 10^{6.5}$ MIPLD₅₀ of RVFV were inoculated were comparable to results obtained in the L cell system (table I) wherein a similar dose was inoculated. At the intermediate dose level a peak titer of $10^{6.2}$ MIPLD₅₀ was obtained on the 4th day; however, by the 7th day only 2+ CPE (50 percent of cells destroyed) was recorded. At the highest dose level a peak titer of $10^{6.8}$ was demonstrated on the 2nd day, but the CPE was not complete until the 7th day.

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The GPL cells were inoculated with $2 \times 10^{6.5}$ and $2 \times 10^{8.5}$ MIPLD₅₀ and incubated at 37 C (table IV). The results were similar to those obtained with the CHL cells (compare tables II and IV).

TABLE III

Growth of Rift Valley fever virus (RVFV) in HeLa cell monolayers at 37 C

Day	Control*	$2 \times 10^{6.5}$ MIPLD ₅₀ †	$2 \times 10^{8.5}$ MIPLD ₅₀ †	$2 \times 10^{6.5}$ MIPLD ₅₀ †	$2 \times 10^{8.5}$ MIPLD ₅₀ †
	CPE‡	Virus/ml§	CPE	Virus/ml	CPE
0		Neg		2.4	3.8
1		2.2		4.0	5.5
2		3.2		5.0	6.8
3		3.3	1+	5.4	6.3
4	1+	3.3	1+	6.2	6.5
5	1+	Trace#	1+	5.2	4.7
6	1+	2.4	1+	5.6	5.8
7	1+	2.0	2+	5.0	6.3

*Control flask—Not inoculated with RVFV. Undiluted medium not lethal for mice (IP).

†Amount of RVFV inoculated per cell culture flask.

‡CPE—Cytopathogenic effect, degree of cell destruction.

§Log₁₀ MIPLD₅₀ of RVFV per ml of cell culture medium (15 ml of medium per flask).

||Neg—No RVFV demonstrated in 1:10 dilution of cell culture medium.

#Trace—Not all of mice died that were inoculated with 1:10 dilution of cell culture medium.

TABLE IV

Growth of Rift Valley fever virus (RVFV) in guinea pig lung (GPL) cell monolayers at 37 C

Day	Control*	$2 \times 10^{6.5}$ MIPLD ₅₀ †	$2 \times 10^{8.5}$ MIPLD ₅₀ †
	CPE‡	Virus/ml§	CPE
0		4.0	3.5
1		7.0	5.7
2		6.8	2+
3	±	7.2	4+
4	±	7.4	4+

*Control flask—Not inoculated with RVFV. Undiluted medium not lethal for mice (IP).

†Amount of RVFV inoculated per cell culture flask.

‡CPE—Cytopathogenic effect, degree of cell destruction.

§Log₁₀ MIPLD₅₀ of RVFV per ml of cell culture medium (15 ml of medium per flask).

Hamster kidney cells were also inoculated with $10^{6.5}$ and $10^{5.5}$ MIPLD₅₀ of RVFV and incubated at 37 C. There was no significant difference in the levels of virus in the culture medium with the two infecting doses used. The peak titer of virus was significantly higher in this culture system than in any of the other four systems. Complete cell destruction also occurred faster than in any of the other systems (table V).

The stability of RVFV in a cell-free cell culture medium (medium 199 plus 10 percent calf serum) is shown by the data presented in table VI.

No difficulty was encountered in passaging RVFV 10 times in CHL and HeLa cell systems. After 10 passages the virus appeared to grow and destroy cells in the same manner as in the first passage. No attempts were made to determine whether there was any change in virulence for either the tissue cultures or the mice after the 10 passages. The virus was not serially passed in the other cell system.

DISCUSSION

The data obtained concerning the susceptibility of various cell culture systems and the information previously available show that RVFV will grow and is cytolytic in a wide variety of tissue or cell culture systems. Further work is indicated to determine which systems are the most suitable for the study of this virus. Effort should be expended to determine whether a tissue culture vaccine similar to that of Randall *et al.*⁹ can be developed and used economically and effectively in livestock.

The dose of virus appeared to influence the time of the peak titer, the level of the peak titer, and the time of maximum or complete cell destruction. Generally, the higher doses resulted in a slightly higher peak titer that occurred at an earlier time than did the titers following the lower doses. There was little difference in the peak titer attained between cultures inoculated with $2 \times 10^{6.5}$ and $2 \times 10^{5.5}$ MIPLD₅₀ of RVFV. Complete cell destruction occurred sooner in those cultures that received the higher doses.

It cannot be elicited from these studies which cell system

would be most suitable for the production and/or assay of this virus. Other dose levels, times of absorption, levels of pH, media, and temperatures of incubation should be considered. Under conditions other than those used in this study the ability of any of the systems to support the growth of

TABLE V

Growth of Rift Valley fever virus (RVFV) in hamster kidney (HK) cell monolayers at 37 C

Day	Control*	2 x 10 ^{5.0} MIPLD ₅₀ †		2 x 10 ^{6.0} MIPLD ₅₀	
	CPE‡	Virus/ml§	CPE	Virus/ml	CPE
0		3.7		2.8	
1		7.4	1+	7.4	1+
2		7.4	4+	7.4	3+
3	±	7.8	4+	8.2	4+

*Control flask—Not inoculated with RVFV. Undiluted medium not lethal for mice (IP).

†Amount of RVFV inoculated per cell culture flask.

‡CPE—Cytopathogenic effect, degree of cell destruction.

§Log₁₀ MIPLD₅₀ of RVFV per ml of cell culture medium (15 ml of medium per flask).

TABLE VI

The stability of Rift Valley fever virus (RVFV) in medium 199 with 10 per cent calf serum at 37 C

Day	Trial 1	Trial 2
0	7.2*	3.6
4	5.8	3.5
8	4.8	3.2
10	4.3	2.5

*Log₁₀ MIPLD₅₀ RVFV per ml of medium.

RVFV may be considerably different from that described here. The hamster kidney system would certainly be of value for the production of virus because of the relatively high titer obtained in a short time. Rosenberger and Shaw¹¹ have shown that several group A and B arthropod-borne viruses grow well on HK cell cultures and that these cell cultures could be used for the titration of the viruses.

SUMMARY

The ability of five lines of cells, L cells, Chang's human liver cells, HeLa cells, guinea pig lung cells, and hamster kidney cells to support the growth of Rift Valley fever virus under a limited set of conditions is described.

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